## CLAIMS

We claim:

5

10

15

20

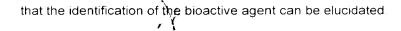
30

35

- 1. An array composition comprising:
  - a) a substrate with a surface comprising discrete sites; and
  - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises:
    - i) a bioactive agent; and
    - ii) an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated;

wherein said microspheres are distributed on said surface.

- 2. An array composition comprising:
  - a) a substrate with a surface comprising discrete sites; and
  - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent and does not comprise an optical signature, wherein said microspheres are distributed on said surface.
- 3 A composition according to claim 1 or 2 further comprising at least one decoder binding ligand.
- 4. A composition according to claim 1 or 2 wherein said bioactive agents are nucleic acids.
- 5. A composition according to claim 1 or 2 wherein said bioactive agents are proteins.
- 25 6 A method of making a composition comprising:
  - a) forming a surface comprising individual sites on a substrate;
  - b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulation each comprising a bioactive agent and do not comprise an optical signature.
  - 7. A method of making a composition comprising:
    - a) forming a surface comprising individual sites on a substrate;
    - b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising:
      - i) a bioactive agent; and
      - ii) an identifier binding ligand that will bind at least one decoder binding ligand such



- $W_{i}$
- 8. A method of decoding an array composition comprising
  - a) providing an array composition comprising:
    - i) a substrate with a surface comprising discrete sites; and
    - ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface;

b) adding a plurality of decoding binding ligands to said array composition to identify the location of at least a plurality of the bioactive agents.

į

10

- 9. A method according to claim 8 wherein at least one subpopulation of microspheres comprises an identifier binding ligand to which a decoding binding ligand can bind.
- 15 10. A method according to claim 8 wherein said decoding binding ligands bind to said bioactive agents.
  - 11. A method according to claim 8 wherein said decoding binding ligands are labeled.
  - 12. A method according to claim 8 wherein the location of each subpopulation is determined.

(رزير) حري

20

- 13. A method of determining the presence of a target analyte in a sample comprising:
  - a) contacting said sample with a composition comprising:
    - i) a substrate with a surface comprising discrete sites; and

25

- ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent and do not comprise an optical signature; wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and
- b) determining the presence or absence of said target analyte.

30

35

- 14. A method of determining the presence of a target analyte in a sample comprising:
  - a) contacting said sample with a composition comprising:
    - i) a substrate with a surface comprising discrete sites; and
    - ii) a population of microspheres comprising at least a first and a second subpopulation each comprising:
      - 1) a bioactive agent; and
      - 2) an identifier binding ligand that will bind a decoder binding ligand such that

the identification of the bioactive agent can be elucidated; wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and

- b) determining the presence or absence of said target analyte.
- 15. An array composition comprising:

5

10

15

- a) a substrate with a surface comprising discrete sites;
- b) a population of microspheres distributed on said sites and comprising at least a first and a second subpopulation, each subpopulation comprising a bioactive agent, wherein said first subpopulation comprises at least a first optical dye with a first pKa and said second subpopulation comprises at least a second optical dye with a second pKa, wherein said first and second pKas are different.
- 16. A method of making a microsphere array comprising:
  - a) contacting a substrate with a surface comprising discrete sites with a solution comprising a population of particles; and
  - b) applying energy to said substrate or said solution, or both, such that at least a subpopulation of said particles associate onto sites.
- 20 17. A method according to claim 16 wherein said discrete sites comprise wells.
  - 18. A method according to claim 16 wherein said energy is in the form of agitation.